After this system has been left for some time at 36°, the foam structure disappears spontaneously and the typical ring structure is restored (fig. 5, $b \rightarrow c$).

It would lead us too far to enter into details concerning the mechanism of the discussed changes, in particular of the shrinking of the original central vacuole. This will be reserved for a later more extensive publication in Protoplasma.

Laboratory for Medical Chemistry at Leiden.

February 1939.

Biochemistry. — *Behaviour of microscopic bodies consisting of biocolloid systems and suspended in an aqueous medium. V. Gelatinized hollow spheres. Temporary invagination to gastrula-like bodies by mechanical or osmotic removal of water from the central cavity.* By H. G. BUNGENBERG DE JONG and O. BANK. (Communicated by Prof. H. R. KRUYT.)

(Communicated at the meeting of February 25, 1939.)

1. In the preceding communication complex coacervate drops were described with a very large central vacuole (liquid hollow spheres, Hohlkörper). These morphological forms have only a limited stability. It is true that the hollow spheres at 36° remain to exist in the surrounding liquid during at least 20 min., but a real equilibrium is out of the question. The volume of the central vacuole is subject to constant changes. Immediately after the formation of the liquid hollow spheres the volume of the vacuole expands, first rapidly, afterwards more slowly, until after a short stationary condition the volume of the vacuole begins to decrease, first slowly, later more rapidly.

Since during these changes the character of hollow sphere is for the present maintained, they must be accompanied by a constant rearrangement of the liquid particles with regard to each other in the skin of coacervate.

In case of a decrease in volume of the vacuole, for example, the thickness of the skin becomes greater and its two globular surfaces smaller (fig. 1A).

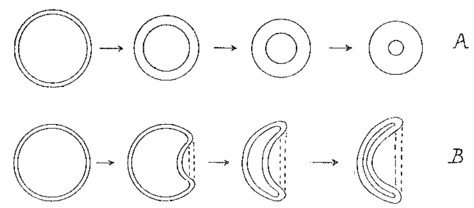


Fig. 1. Removal of liquid from the vacuole in a liquid (A) and a gelatinized (B) hollow sphere. It is supposed that the volume of the coacervate and of the gel during this withdrawal of liquid remains constant.

In this communication we shall discuss a few characteristics of the gel bodies, obtained by cooling of the liquid hollow spheres, in particular the changes they display upon removal of liquid from the central cavity.

Owing to the change of the coacervate into a gel, in these gel bodies rearrangements of the particles with regard to each other, as were possible during the liquid state in the coacervate skin, are not or only to a very slight extent possible in the globular skin of gel which has now been formed.

It is to be expected that, if withdrawal of liquid from the vacuole takes place, the gelatinized skin cannot remain globular. The deformation occurring here is an invagination, unless by forcible means another form of deformation is prescribed (fig. 1B).

This removal of vacuolar liquid may be achieved in two ways:

a. by mechanical means,
b. by an osmotic process.

2. *Invagination after a mechanical pressure and spontaneous reversal of the invagination.*

If a suspension of gelatinized hollow spheres is brought on an object-glass and covered with a cover-glass, it is already sufficient to turn the tube of the microscope down, so that the objective presses against the cover-glass, and to make this pressure last for some seconds to observe invagination in many hollow spheres after the tube has been turned up. After the preparation has been left for some time, it appears that the invagination gradually decreases and finally vanishes completely, the original form of the hollow sphere being restored. We can also put a pressure upon the cover-glass in another way and at the same time continue the examination under the microscope. We find then that the invagination does not set in during the pressure but after this has been removed. Then it takes place suddenly.

In order to be able to explain this (see fig. 2), we have to take into consideration that the gelatinized hollow spheres are elastic. During the

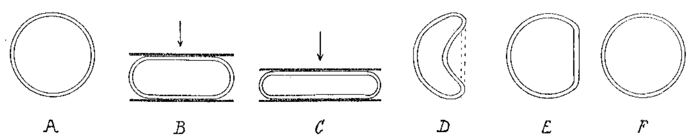
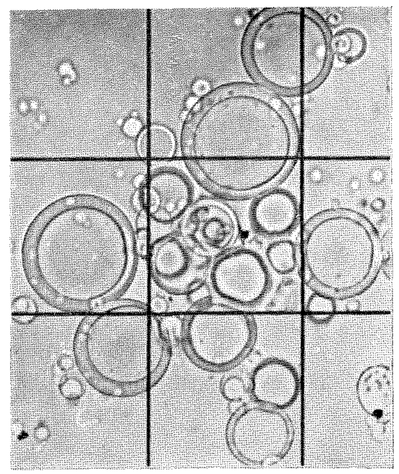


Fig. 2. Reversible invagination by mechanical pressure.
A. original condition.
B and C. deformation between two planoparallel plates.
In B, immediately after application of the pressure, the volume of the vacuole is still equally large as in A; in C, after continued pressure, a considerable amount of liquid has been pressed out.
D. after the pressure has been removed, deformation C changes into an invagination.
E. stage during the spontaneous reversal of the invagination.
F. final stage.

pressure, while water is pressed from the vacuole ($B \rightarrow C$), an elastic deformation of the hollow sphere is brought about, invagination not yet playing a part. After the pressure has been removed, the ring body tries to recover its original shape, but this is impossible, the content of the vacuole now being smaller. However, the deformation produced under

H. G. BUNGENBERG DE JONG AND O. BANK: BEHAVIOUR OF MICROSCOPIC BODIES CONSISTING OF BIOCOLLOID SYSTEMS AND SUSPENDED IN AN AQUEOUS MEDIUM. V. GELATINIZED HOLLOW SPHERES. TEMPORARY INVAGINATION TO GASTRULA-LIKE BODIES BY MECHANICAL OR OSMOTIC REMOVAL OF WATER FROM THE CENTRAL CAVITY.



A (160 ×)

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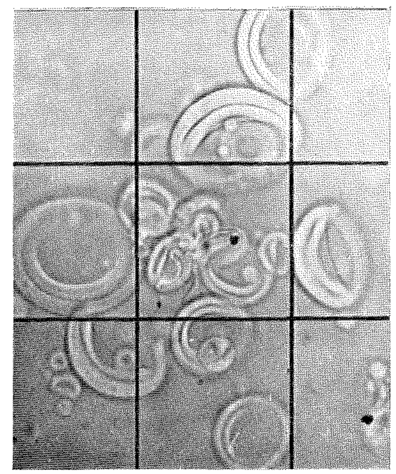

B (160 ×)

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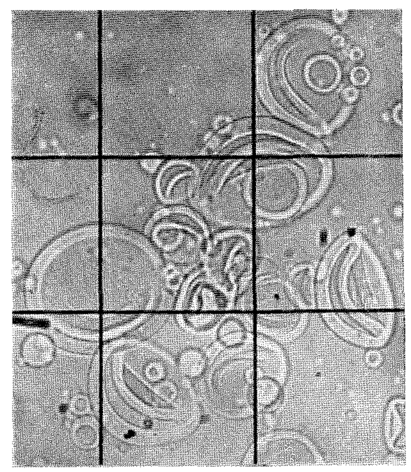

C (160 ×)

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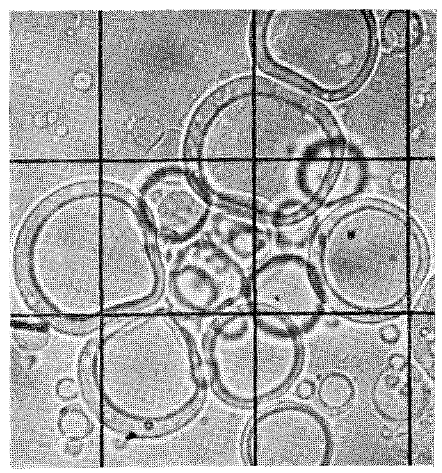

D (160 ×)

pressure may spontaneously change into another deformation with the same volume of the vacuole, if this required less deformation energy for its formation starting from the hollow sphere. This deformation is the one where folding (invagination) of part of the globular skin has taken place. In this new condition D the elastic stretching, existing in C in the upper and lower half of the globular skin, has disappeared and the length of the zone with a strong curvature is considerably smaller.

The condition now obtained (D) is still one where an elastic deformation exists and consequently should not be stable. From now onwards, under the influence of the existing elastic deformation, liquid must flow from outside into the vacuole, thus enabling the elastic deformation to decrease. This is indeed the case. Gradually the folding is growing less deep and finally we notice that it disappears completely, the original form being restored (fig. 2F). The last rounding ($E \rightarrow F$) usually takes place with surprising rapidity.

3. *Osmotic behaviour of the gelatinized hollow spheres.*

If we bring a suspension of the gelatinized hollow spheres on an object-glass and leave this for some time, they stick to the glass surface, so that upon addition of a drop of aqueous solution most of them remain in their places. The subjoined microphotographs (distance between 2 black lines = 130 μ) refer to the influence of cane-sugar. A photograph was taken of part of the preparation without cover-glass (A), then a drop of 30 % cane-sugar solution was carefully placed on it and a second photograph was taken (B). We see that a very strong invagination has set in. Twenty minutes afterwards once more a photograph was taken (C). Evidently the intensity of the invagination has distinctly decreased. In the large objects the distance between the convex and concave part of the globular skin is distinctly larger, while some small objects, invaginated in B, in C have become round again.

The described changes may be regarded as the result of a temporary osmotic withdrawal of water from the central cavity, owing to the fact that the permeability of the wall for water is evidently considerably greater than for sugar. After the invagination has set in, no osmotic equilibrium is attained, since cane-sugar also diffuses slowly inwards. Consequently the intensity of invagination decreases gradually.

This spontaneous reversal of the invagination ultimately leads to a complete restoration to the original blastula form. Since in the case of cane-sugar in large objects this process may take a long time, it was accelerated by addition of a fairly large quantity of distilled water (D). We now observe yet another phenomenon, viz. that after neutralization of the invagination the hollow spheres now are larger and the globular gel skins thicker than at the beginning of the experiment.

Owing to the fact that the pH was not held constant, this general increase in size may be attributed mainly to a swelling of the gel skin as a result of an increase of pH of the medium (the intensity of the complex relations between positive gelatin and negative gum arabic decreases).

The phenomena discussed above may be realized not only with cane-sugar but also with solutions of other substances, with non-electrolytes as well as with electrolytes. Here distinct differences occur in the time after which, by spontaneous reversal of the invagination, complete rounding is again recovered.

Although quantitative experiments have not yet been made, we are inclined to conclude from qualitative experiments that the permeability of the globular gel skin increases in the order

$$\text{cane-sugar} < \text{glycerin} < \text{alcohol}.$$

With urea invagination may be obtained as well, but now the images are difficult to see and secondary changes are apt to occur, owing to the fact that in this medium the gelatination decreases or is neutralized. As a rule the duration of the invagination produced with salt solutions is only short. This striking difference between salts (e.g. NaCl) and non-electrolytes (cane-sugar, etc.) may for the rest be explained by the secondary reactions by which the former do, the latter do not affect the complex gel of which the globular gel skin consists. Salts, namely, reduce the intensity of the complex relations between positive gelatin and negative gum arabic (in analogy with the neutralizing action of salts on the complex coacervates). The result of the salt reaction is an increasing water content of the complex gel, which makes it conceivable that the permeability of the globular gel skin for salts is much more considerable than for non-electrolytes.

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